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HYPERTENSION AND 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN IN AIR FORCE VETERANS OF THE VIETNAM WAR

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14. ABSTRACT We studied the risk of hypertension and exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in veterans of Operation Ranch Hand, the Air Force unit responsible for the aerial spraying of Agent Orange and other TCDD-contaminated herbicides in Vietnam. We included a Comparison group of other Air Force veterans who flew or serviced C-130 cargo aircraft in Southeast Asia during the same calendar period that the Ranch Hand unit was active in Vietnam (1962-1971) but were not involved with spraying herbicides. We measured TCDD serum level in 1987, 1992, and 1997. There was no overall increase in the risk of hypertension in the Ranch Hand cohort, however, within both cohorts, the risk of hypertension was markedly increased with TCDD. Relative to the bottom category of serum TCDD in the Comparison cohort, the relative risk of hypertension in the highest TCDD category in the Comparison group was 1.66 (95% CI 1.04 to 2.67) and 1.33 (1.02 to 1.74) in the highest TCDD category in the Ranch Hand group. An analysis of a questionnaire-based index of skin exposure to herbicides among Ranch Hand enlisted personnel revealed an increasing trend (p=0.002) of hypertension risk with increasing skin exposure. While the lack of an overall between-group difference in hypertension risk suggested that TCDD was not a risk factor for hypertension, these within-group associations suggested that mechanisms relating TCDD uptake and clearance were associated with body weight and the pathphysiology of hypertension.

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Hypertension and 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Air Force Veterans of the Vietnam War

by

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9 February 2006

Abbreviations

Abbreviation	Definition
BMI	Body Mass Index
ICD	International Classification of Diseases
kg	Kilogram
log	Logarithm
m	Meter
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

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ABSTRACT

We studied the risk of hypertension and exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in veterans of Operation Ranch Hand, the Air Force unit responsible for the aerial spraying of Agent Orange and other TCDD-contaminated herbicides in Vietnam. We included a Comparison group of other Air Force veterans who flew or serviced C-130 cargo aircraft in Southeast Asia during the same calendar period that the Ranch Hand unit was active in Vietnam (1962-1971) but were not involved with spraying herbicides. We measured TCDD serum level in 1987, 1992 and 1997. There was no overall increase in the risk of hypertension in the Ranch Hand cohort, however, within both cohorts, the risk of hypertension was markedly increased with TCDD. Relative to the bottom category of serum TCDD in the Comparison cohort, the relative risk of hypertension in the highest TCDD category in the Comparison group was 1.66 (95% CI 1.04 to 2.67) and 1.33 (1.02 to 1.74) in the highest TCDD category in the Ranch Hand group. An analysis of a questionnaire-based ind-ex of skin exposure to herbicides among Ranch Hand enlisted personnel revealed an increasing trend (p=0.002) of hypertension risk with increasing skin exposure. While the lack of an overall between-group difference in hypertension risk suggested that TCDD was not a risk factor for hypertension, these within-group associations suggested that mechanisms regulating TCDD uptake and clearance were associated with body weight and the pathophysiology of hypertension.

Key words: Agent Orange, dioxin, epidemiology, hypertension, TCDD

INTRODUCTION

Polychlorodibenzo-p-dioxins (PCDDs) and -furans (PCDFs) are ubiquitously distributed toxic lipophilic substances, can be detected in human serum lipid and adipose tissue, and are slowly eliminated. The primary source of human uptake is via food, especially fish and dairy products. The PCDD 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been the focus of many epidemiological and toxicological studies throughout the world. Measurements of TCDD in specimens collected in 1982 from 763 individuals (composited into 46 samples) in the United States found mean (SEM) levels, in parts per trillion (ppt), ranging from 4.1 (1.2) in the West to 7.1 (1.6) in the North Central region[1]. More recent studies suggest that mean concentrations in the general population have been declining[2].

Relatively few data exist on the effects of TCDD exposure on hypertension in humans, and findings across studies are inconsistent[3-4]. Following an explosion at a chemical plant in 1976, residents of Seveso, Italy, were exposed to sodium hydroxide, ethylene glycol, the sodium salt of 2,4,5-trichlorophenol, and kilogram amounts of TCDD[3]. Primarily on the basis of animal and vegetation mortality and TCDD measurements in soil and grass, the contaminated area was divided into three zones, designated A, B, and R. Zone A covered 87 hectares and the mean concentration in soil ranged between 15.5 $\mu g/m^2$ and 580.4 $\mu g/m^2$; Zone B covered 270 hectares and mean TCDD in soil ranged up to 50 $\mu g/m^2$; zone R covered 1430 hectares where TCDD in soil ranged up to 5 $\mu g/m^2$. Measurements of serum TCDD in 1976 from selected adults in

zones A, B and R were made; the median in zone A was 443 ppt (range: 1.5 ppt to 10,400 ppt); the median in zone B was 94 ppt (range: 1 ppt to 725 ppt); the median in zone R was 48 ppt (range: 1 ppt to 545 ppt). A mortality study through the end of 1991[3] found a significant increase in the relative risk (RR) of death caused by hypertension among women (person-years=5975, deaths=3, RR=3.6, 95% CI 1.2 to 11.4) and no significant increase among men (person-years=5541, deaths=1, RR=2.3, 95% CI 0.3 to 16.5) in zone A; no significant increases were found in men or women from zones B or R. Workers exposed to TCDD during the production of trichlorophenol or one of its derivatives at one of two plants in Newark, New Jersey, and Verona, Missouri, between 1951 and 1969 (n=400), were physically examined in 1987; serum measurements of TCDD made in 1987 ranged from non-detectable levels to 3,400 ppt[4]. Workers were stratified to those with TCDD concentrations less than 138 ppt (n=208) and those with TCDD levels at least 138 ppt (n=65). Contrasts with matched neighborhood referents found no significant increase in the risk of self-reported physician-diagnosed hypertension in the lower (RR=1.14, 95% CI 0.79 to 1.66) or the higher (RR=1.46, 95% CI 0.84 to 2.67) TCDD exposure category.

We examined hypertension in relation to TCDD measured in veterans participating in the Air Force Health Study, an epidemiological investigation of veterans of Operation Ranch Hand, the unit responsible for the aerial spraying of herbicides, including Agent Orange, in Vietnam from 1962 to 1971. Agent Orange was a 1:1 mixture of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxy-acetic acid and was contaminated, from less than 0.05 to almost 50 parts per million, with TCDD[5]. Data

used in this paper were gathered between 1982 and 1998 from Air Force veterans whose exposure in Vietnam occurred up to 35 years earlier.

MATERIALS AND METHODS

The Air Force Health Study is an ongoing 20-year prospective epidemiologic study that seeks to determine whether veterans of Operation Ranch Hand (the Air Force personnel who conducted aerial herbicide spray operations during the Vietnam War) have experienced adverse health that can be attributed to exposure to herbicides or their TCDD contaminant. Details of the study design and subject selection are described elsewhere[6]. Exposure to herbicides could have occurred during maintenance of the aircraft and herbicide spray equipment or during flight operations. A Comparison group of other Air Force veterans who served in Southeast Asia (SEA) during the same period that the Ranch Hand unit was active but who were not involved with spraying herbicides was included. Comparison veterans were matched to Ranch Hand veterans with respect to age, race and military occupation. All study subjects were male. Physical examinations were performed in 1982, 1985, 1987, 1992 and 1997. Participation was voluntary and informed consent was given at the examination sites. The study includes assessments of the health[7-11], mortality experience[12,13] and reproductive outcomes[14-17] of Ranch Hand veterans. To date (2001), results suggest a relation between TCDD levels and diabetes[7-8], an increased risk of death caused by cardiovascular disease in enlisted ground crew[12], no evidence of chloracne (an acne-like condition associated with acute exposure to TCDD)[9], no relation between TCDD and cancer prevalence[10] or immune function[11], no relation

between TCDD levels and sperm count or sperm abnormalities[14], and little or no relation between paternal TCDD levels and miscarriage[15], birth defects[15], specific delays in development[15], low birth weight[16], pre-term birth[16], or the gender of offspring[17]. The statistical power of this study is limited by the size of the Ranch Hand group and the modest level of exposure compared with subjects in zone A or B of Seveso[18] or in selected occupationally exposed cohorts[4, 19-22].

We report analyses of hypertension and TCDD body burden among veterans who participated in the 1982, 1985, 1987, 1992 and 1997 studies. Beginning in 1987, blood from willing veterans was collected and TCDD was measured in serum at the Centers for Disease Control and Prevention and expressed as ppt in serum lipid[23]. The serum TCDD measurements were done with high-resolution gas chromatography/high-resolution mass spectrometry[24]. The between assay coefficient of variation at three different concentrations of TCDD ranged from 9.4% to 15.5%.

TCDD concentrations were measured on a lipid weight basis in serum[24,25] collected from veterans who completed the 1987 physical examination. Additional measurements were made in 1992 and 1997. For those veterans whose TCDD level was not measured in 1987, the subsequent measure was extrapolated to 1987 using a first-order kinetics model with a constant half-life of 7.6 years[26]. Non-detectable (non-quantitatable) TCDD levels were replaced by the value of the limit of detection (limit of quantitation) divided by $\sqrt{2}$ [27].

At each examination each participant was asked if a doctor had ever told him that he had hypertension, and a physician made blood pressure measurements. All reported conditions were verified by medical record review and were coded according to the International Classification of Diseases, 9th Edition, Clinical Modification (ICD-9-CM)[28]. We studied diagnosed hypertension (ICD 401) that occurred after service in Southeast Asia and prior to April 1999. We did not study blood pressure measurements because many study participants had taken or were taking medications to control their blood pressure.

We excluded veterans with no TCDD result and those with diagnosed hypertension prior to their service in SEA. Missing TCDD levels were caused by refusal, noncompliance to the physical examination, death, or a failure of one or more quality control checks and insufficient sample to repeat the analysis. Because preliminary statistical analyses found hypertension to be associated with TCDD within both cohorts, we present data summaries within both groups by category of TCDD serum concentration. To this end, we defined 4 TCDD categories in the Comparison cohort, labeled C1 through C4, and 6 categories in the Ranch Hand cohort, labeled R1 through R6. The first 3 categories in each cohort were defined by TCDD tertiles among all veterans with TCDD less than or equal to 10 ppt, a value we have used as a threshold for background exposure[7]. The 4th TCDD category among Comparison veterans was comprised of veterans with TCDD greater than 10 ppt. The 4th, 5th and 6th TCDD categories among Ranch Hand veterans were determined by TCDD tertiles among those

with TCDD greater than 10 ppt. Both the sample reduction and the net sample sizes by TCDD category and cohort are summarized in Table 1.

Table 1. Sample reduction, TCDD category ranges, and hypertension in 2,417 US Air Force Vietnam veterans.

Cohort	TCDD Category: [Range†]	N	Hypertension prior to service in SEA‡	Net	Hypertension (%) after service in SEA‡
Comparison		1,571*	(21)	1,550	
No TCDD result		135	(0)	135	
Net		1,436	(21)	1,415	580 (41.0)
	C1: [0.42 to 3.33]	538	(7)	531	169 (31.8)
	C2: [3.34 to 5.16]	504	(5)	499	158 (38.9)
	C3: [5.17 to 9.97]	360	(9)	351	172 (47.3)
	C4: [10.1 to 54.8]	34	(0)	34	21 (61.8)
Ranch Hand		1,111*	(14)	1,097	
No TCDD result		95	(0)	95	
Net		1,016	(14)	1,002	405 (40.4)
	R1: [0.57 to 3.33]	76	(0)	76	16 (21.1)
	R2: [3.38 to 5.14]	112	(2)	110	34 (30.9)
	R3: [5.18 to 10.00]	254	(3)	251	99 (39.4)
	R4: [10.02 to 17.6]	191	(5)	186	77 (41.4)
	R5: [17.7 to 36.8]	192	(2)	190	91 (47.9)
	R6: [37.0 to 617.8]	191	(2)	189	88 (46.6)
Net		2,452	(35)	2,417	984 (40.7)

*Compliant to at least one of five physical examinations (in 1982, 1985, 1987, 1992 or 1997).
†Parts per trillion.
‡Southeast Asia.

Statistical analyses

We analyzed hypertension against both discrete and continuous measurements of TCDD using Cox proportional hazards models[29]. The Cox models were stratified by year of birth in 5-year intervals (≤1920, 1921-1925, 1926-1930, 1931-1935, 1936-1940, 1941-1945, 1946-1950). The time-to-onset of hypertension was the time (in years) from the end of service in Southeast Asia to the first diagnosis of hypertension. The time-to-onset of those veterans without a history of hypertension was censored at the date of the last physical examination attended. If a veteran died after his last examination and there was no mention of hypertension on the death certificate and no prior diagnosis of hypertension, then the time-to-onset was censored at his date of death.

We computed body mass index (BMI) as weight (kg) divided by the square of height (m^2). We defined a pack-year as smoking one pack of cigarettes per day for one year and a drink-year as drinking one shot of 80-proof whiskey (or, equivalently, 12 ounces of beer or 5 ounces of wine) per day for one year. Pack-years and drink-years were estimated as of the 1982 physical examination based on questionnaires administered at the exams. Covariate adjustments were made for age at the end of service in SEA, race (Black, nonblack), military occupation (officer, enlisted flyer, enlisted ground crew), BMI at the end of service in SEA, the change in BMI from the end of service in SEA to the TCDD measurement, lifetime cigarette smoking history up to the baseline physical examination in 1982 (pack-years), lifetime drinking history up to the baseline physical examination in 1982 (drink-years), serum lipids (total cholesterol, HDL cholesterol, and triglycerides) measured in the serum used for the TCDD measurement, and family history

of hypertension (yes, no) in first degree relatives (parents or siblings). Family history of hypertension was recorded as "yes" only if a parent was reported as having had hypertension before the veteran reached the age of 40 or a sibling was reported as having hypertension before the veteran reached age 70.

First, using the TCDD categories defined in Table 1, we applied a Cox proportional hazards model to estimate the relative risk of hypertension in the 2nd, 3rd, and 4th TCDD categories in the Comparison group (C2 through C4) and all six TCDD categories in the Ranch Hand group (R1 through R6) relative to the 1st TCDD category in the Comparison group (C1).

Second, we used Cox proportional hazards models to estimate the relative risk of hypertension per unit change in logarithm base 2 of TCDD within each cohort. This second group of Cox models had the advantage that the beta-coefficient was directly interpretable as the logarithm of relative risk corresponding to a doubling of the TCDD concentration. To account for unequal within cohort beta-coefficients, the overall between cohort relative risk was assessed at TCDD equal to 4 ppt. Plots of hypertension prevalence and BMI averages versus TCDD category are included.

In an earlier paper[30] we found that the TCDD was related by a regression model to both BMI in Vietnam and an index of skin exposure to herbicides based on responses to a questionnaire administered to enlisted Ranch Hand veterans prior to any veteran knowing

his serum TCDD value. The index was the estimated total number of days of the Vietnam tour during which herbicide came into contact with skin, and was computed using the reported number of months each duty was performed and how often it was performed during each month. To assess whether associations between hypertension and TCDD were due solely to confounding with BMI, we performed a series of secondary analyses using the skin exposure index instead of TCDD among enlisted Ranch Hand veterans. Omitted from statistical analyses were veterans with an administrative job classification who were older, heavier, and may have had less herbicide exposure than their subordinates.

In these secondary analyses we first regressed the logarithm base 2 of TCDD on military occupation (enlisted flyers, enlisted ground crew), the logarithm of the skin exposure index, BMI at the end of service in SEA, and the square of BMI at the end of service in SEA. We used the results from this regression to plot values of BMI and the skin exposure index that predicted a constant value (in this case the median) of TCDD in enlisted Ranch Hand veterans.

Next, we categorized the skin exposure index by quintiles of the distribution and fitted Cox proportional hazards models to estimate the relative risk of hypertension in the 2^{nd}, 3^{rd}, 4^{th} and 5^{th} quintiles relative to the 1^{st} quintile. Year of birth was stratified in 5-year intervals (≤1925, 1926-1930, 1931-1935, 1936-1940, 1941-1945, 1946-1950). Covariate adjustments were otherwise similar to those for the analyses of TCDD categories, except that we additionally adjusted for the ratio of the skin exposure index to

the total number of days of each veteran's tour in Vietnam as determined from a review of his military records. We conducted two-sided testing with a significance level of 5% throughout and used SAS® software (SAS Institute, Carey, NC) for all analyses and graphics.

RESULTS

Table 2 summarizes TCDD levels, demographics, lipids, and family history of hypertension in each cohort. The median TCDD in the Ranch Hand group (11.6 ppt) was significantly greater than the median (4.0 ppt) in the Comparison group ($p<0.001$). The mean (SD) BMI (kg/m^2) during service in Vietnam was 24.9 (3.0) in Comparisons and 24.7 (3.0) in Ranch Hand veterans; the 95^{th} percentile was 30.1 in Comparisons and 29.8 in Ranch Hand veterans. Overall, the two groups were similar with regard to measured demographic attributes, partly due to the matching on age, race and military occupation. The mean total cholesterol, HDL cholesterol, triglycerides, and the percentage with a family history of hypertension or stroke were also similar in both groups.

Table 2. Distribution of TCDD, demographic characteristics, and lipids, in US Air Force Vietnam veterans.

Group	TCDD* Median (Range)	Age† Mean (Std)	Race (% Black)	BMI† (kg/m^2) Mean (Std)	Change‡ in BMI (kg/m^2) Mean (Std)
Comparison	4.0 (0.4 to 55)	30.2 (7.4)	6.5	24.9 (3.0)	2.9 (3.1)
Ranch Hand	11.6 (0.6 to 618)	29.8 (7.3)	5.9	24.7 (3.0)	2.9 (3.1)

Group	Officer (%)	Enlisted Flyer (%)	Enlisted Ground Crew (%)	Reported Family History of Hypertension (%)
Comparison	38.2	15.5	46.4	31.3
Ranch Hand	37.5	17.4	45.1	33.4

Group	Cigarette Smoking to 1982 (Pack-years) Median (Range)	Drinking to 1982 (Drink-years) Median (Range)
Comparison	9.4 (0 to 104)	12.4 (0 to 627)
Ranch Hand	11.3 (0 to 139)	11.6 (0 to 518)

Group	Total Cholesterol§ (mg./dl)	HDL Cholesterol§ (mg/dl)	Triglycerides§ Median (Range)
Comparison	217.5 (39.6)	46.2 (12.2)	119.0 (22 to 1500)
Ranch Hand	219.3 (39.8)	46.5 (12.4)	116.5 (14 to 2130)

*Parts per trillion, measured in 1987, 1992 or 1997.
†At the end of service in Southeast Asia.
‡From end of service in Southeast Asia to TCDD measurement.
§In the serum specimen used for the TCDD measurement.

Two TCDD exposure categories were defined as below or above 5 ppt (the approximate Comparison mean TCDD concentration) and hypertension risk was summarized by TCDD category within groups, without adjustment for known risk factors. The unadjusted relative risk between groups, ignoring this TCDD dichotomy, was also determined (Table 3). Within each group, the risk of hypertension was significantly increased among veterans with TCDD at least 5 ppt (Comparison: RR=1.39, 95% CI 1.23 to 1.57, p<0.0001, Ranch Hand: RR=1.62, 95% CI 1.26 to 2.10, p<0.0001). These two relative risks were not significantly different (p=0.52) and the Mantel-Haenszel estimate of the common relative risk was significantly increased (RR=1.45, 95% CI 1.29 to 16.3, p<0.0001). However, ignoring the TCDD dichotomy, the relative risk between groups was not significantly increased (RR=1.01, 95% CI 0.92 to 1.12, p=0.78).

Table 3. Relative risk of hypertension by TCCD level and group.

Group	TCDD category	N	Hypertension (%)	RR (95% CI)	p-value
Comparison	[0, 5.0]	1000	368 (36.8)		
	[5.0 ,54.8]	415	212 (51.1)	1.39 (1.23, 1.57)	<0.0001
Ranch Hand	[0, 5.0]	176	47 (26.7)		
	[5.0, 617.8]	826	358 (43.3)	1.62 (1.26, 2.10)	<0.0001
Pooled*	[0, 5.0]				
	[5.0, 617.8]			1.45† (1.29, 1.63)	<0.0001
Comparison		1415	580 (41.0)		
Ranch Hand		1002	405 (40.4)	1.01 (0.92, 1.12)	0.78

* Test for homogeneity of odds ratios: p=0.52.

† Mantel-Haenszel estimate of the common relative risk.

Table 4 summarizes the relative risk of hypertension by category of serum TCDD in the Comparison and Ranch Hand cohorts separately, with the lowest category of the Comparison group as the referent, for each of three statistical models: 1) adjusted only for year of birth; 2) adjusted for year of birth, military occupation, race, age and BMI at the end of service in Vietnam and family history of hypertension; and 3) adjusted for year of birth, military occupation, race, age and BMI at the end of service in SEA, drink-years, pack-years, change in BMI from the end of service in SEA to the TCDD measurement, lipids (total cholesterol, HDL cholesterol and triglycerides in the serum used for the TCDD measurement), and family history of hypertension. The relative risks were significantly increased in the highest two categories of both the Comparison and in Ranch Hand groups in all three models.

Table 4. Relative risk of hypertension by category of TCDD relative to the first TCDD category in the Comparison cohort.

TCDD Category	Model 1* RR (95% CI)	Model 2† RR (95% CI)	Model 3‡ RR (95% CI)
		Comparison Group	
C1: [0.42 to 3.33]	Reference for both groups		
C2: [3.34 to 5.16]	1.34‖ (1.09 to 1.64)	1.24§ (1.01 to 1.52)	1.19 (0.97 to 1.47)
C3: [5.17 to 9.97]	1.70¶ (1.37 to 2.11)	1.46¶ (1.17 to 1.81)	1.38‖ (1.11 to 1.73)
C4: [10.1 to 54.8]	2.46¶ (1.56 to 3.88)	2.07‖ (1.31 to 3.28)	1.66§ (1.04 to 2.67)
Trend#	p<0.001	p=0.001	p=0.022
		Ranch Hand Group	
R1: [0.57 to 3.33]	0.59§ (0.36 to 0.99)	0.77 (0.46 to 1.29)	0.83 (0.50 to 1.40)
R2: [3.38 to 5.14]	0.79 (0.55 to 1.15)	0.92 (0.63 to 1.34)	0.95 (0.65 to 1.38)
R3: [5.18 to 10.00]	1.20 (0.93 to 1.54)	1.22 (0.95 to 1.57)	1.26 (0.98 to 1.63)
R4: [10.02 to 17.6]	1.19 (0.91 to 1.56)	1.11 (0.84 to 1.46)	1.12 (0.85 to 1.47)
R5: [17.7 to 36.8]	1.66¶ (1.29 to 2.15)	1.47‖ (1.13 to 1.90)	1.38§ (1.06 to 1.79)
R6: [37.0 to 617.8]	1.71¶ (1.32 to 2.22)	1.40§ (1.07 to 1.83)	1.33§ (1.02 to 1.74)
Trend#	p<0.001	p=0.006	p=0.032

*Adjusted only for year of birth.

†Adjusted for year of birth, military occupation, race, age and BMI at the end of service in Southeast Asia, and family history of hypertension. Stratified by year of birth in 5 year intervals.

‡Adjusted for year of birth, military occupation, race, age and BMI at the end of service in Southeast Asia, family history of hypertension, alcohol drink-years to 1982, smoking pack-years to 1982, change in body mass index from the end of service in Southeast Asia to the TCDD measurement, and lipids (total cholesterol, HDL cholesterol and triglycerides in the serum used for the TCDD measurement). Stratified by year of birth in 5 year intervals.

§ Significantly different from 1.0 ($p<0.05$).

‖ Significantly different from 1.0 ($p<0.01$).

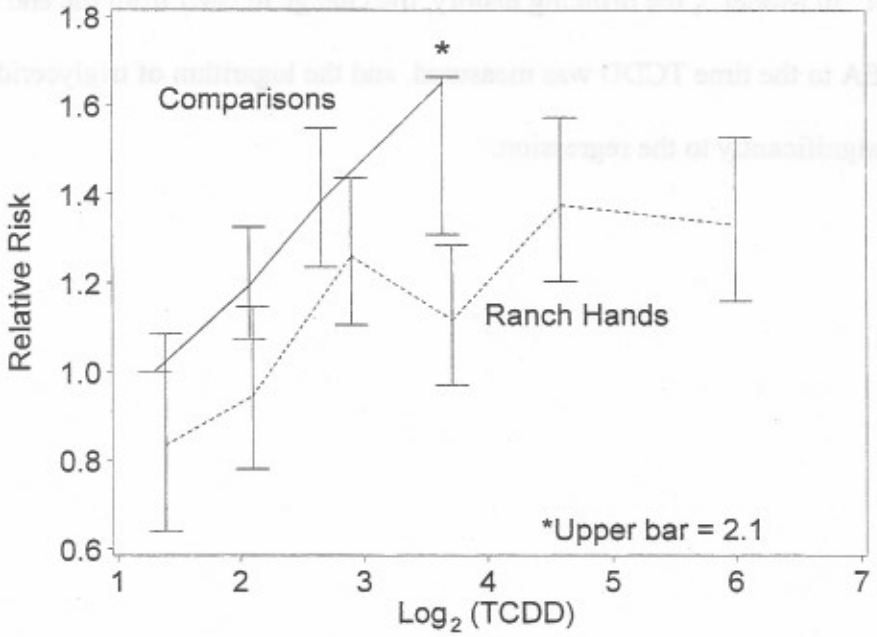
¶ Significantly different from 1.0 ($p<0.001$).

P-value of linear trend test on relative risks within Comparison group and Ranch Hand group.

Compared to the unadjusted relative risks (Model 1 in Table 4), adjustment for potential confounding or mediating factors (Models 2 and 3 in Table 4) reduced, but did not eliminate the TCDD gradient in both cohorts. For all three models, the relative risks of hypertension increased significantly within both cohorts as TCDD increased.

The relative risk of hypertension from Model 3 is depicted by TCDD category and cohort in Figure 1, with the median serum TCDD level within category plotted on the x-axis. The gradient of increasing risk across serum TCDD quintiles was similar within the Comparison and Ranch Hand cohorts, even though the levels of serum TCDD were substantially increased in Ranch Hand relative to Comparison veterans.

Figure 1. Relative risk of hypertension versus TCDD category median by exposure cohort



LEGEND: The median of the logarithm base 2 of TCDD within each TCDD category is plotted on the horizontal axis. The estimated Cox model risk of hypertension is relative to the first category of the Comparison group. Error bars represent the relative risk plus or minus one standard error of the relative risk. Relative risk is adjusted for year of birth, military occupation, race, age at the end of service in Southeast Asia, and BMI at the end of service in Southeast Asia, family history of hypertension, alcohol drink-years to 1982, smoking pack-years to 1982, change in body mass index from the end of service in Southeast Asia to the TCDD measurement, and lipids (total cholesterol, HDL cholesterol and triglycerides in the serum used for the TCDD measurement).

Table 5 shows the regression coefficients and p-values for the covariates used in Models 2 and 3 for the analyses summarized in Table 4. Particularly significant in both Models 2 and 3 were age and BMI at the end of service in SEA and a family history of hypertension. In Model 3, the drinking history, the change in BMI from the end of service in SEA to the time TCDD was measured, and the logarithm of triglycerides contributed significantly to the regression.

Table 5. Cox regression coefficients for covariates from Models 2 and 3 in Table 3.

Variable	Model 2 Regression Coefficient (Std Error)	p-value	Model 3 Regression Coefficient (Std Error)	p-value
Enlisted flyer	0.1229 (0.0971)	0.21	0.1078 (0.0987)	0.27
Enlisted ground crew	0.1257 (0.0844)	0.14	0.0840 (0.0862)	0.33
Race*	0.1947 (0.1275)	0.13	0.2956 (0.1298)	0.02
Age†	0.0434 (0.0149)	0.004	0.0569 (0.0151)	<0.001
Body mass index†	0.0844 (0.0110)	<0.001	0.0798 (0.0112)	<0.001
Family history of hypertension	0.4529 (0.0690)	<0.001	0.4410 (0.0692)	<0.001
Drinking history¶‖			0.0211 (0.0058)	<0.001
Smoking history#			0.0005 (0.0018)	0.78
Change in body mass index‡			0.0605 (0.0099)	<0.001
Total Cholesterol§			-0.0003 (0.0009)	0.71
HDL cholesterol§			0.0050 (0.0031)	0.11
Triglycerides§‖			0.3688 (0.0625)	<0.001

*Black=1, nonblack=0.
† At the end of service in Southeast Asia.
‡ From the end of service in Southeast Asia to the TCDD measurement.
§ In the serum used for the TCDD measurement.
‖ Log transformed.
¶Drink-years to 1982 divided by 10.
#Pack-years to 1982.

Table 6 summarizes results from the second set of Cox proportional hazards models. These models were similar to those already presented in Table 5, except that we replaced the TCDD categories by an overall cohort indicator (Ranch Hand, Comparison) and the logarithm (base 2) of serum TCDD levels within each cohort separately. The between cohort relative risk at a TCDD of 4 ppt was significantly reduced (RR=0.70, 95% CI 0.58 to 0.83, $p<0.001$) for Model 1, but was attenuated by adjustment for confounding and mediating covariates in Model 3 (RR=0.90, 95% CI 0.74 to 1.09, $p=0.26$). Within both cohorts serum TCDD was a strong predictor of hypertension risk in both Models 1 and 2. Although somewhat attenuated relative to Model 1, the Model 2 relative risks were increased for Comparison (RR=1.33, 95% CI 1.20 to 1.48, $p<0.001$) and for Ranch Hand (RR=1.09, 95% CI 1.02 to 1.17, $p=0.010$) veterans. After the addition of more covariates in Model 3, the relative risk of TCDD within the Comparison cohort was still increased (RR=1.28, 95 CI 1.15 to 1.43, $p<0.001$), while it was attenuated (RR=1.07, 95 CI 1.00 to 1.15, $p=0.054$) in the Ranch Hand cohort. Tests for cohort differences with regard to within-cohort coefficients relating hypertension and $\log_2$(TCDD) were significant ($p<0.01$) for all three models, with the Comparison coefficient being greater than the Ranch Hand coefficient in each model

Table 6. Relative risk of hypertension given that TCDD doubles, based on regression where logarithm (base 2) of TCDD is entered as a continuous variable within the Comparison and Ranch Hand groups.

Contrast or variable	Model 1* RR (95% CI)	p-value	Model 2† RR (95% CI)	p-value	Model 3‡ RR (95% CI)	p-value
Between group Difference§	0.70 (0.58 to 0.83)	<0.001	0.84 (0.70 to 1.01)	.066	0.90 (0.74 to 1.09)	0.262
Log_2 (TCDD) within Comparison cohort	1.44 (1.29 to 1.61)	<0.001	1.33 (1.20 to 1.48)	<0.001	1.28 (1.15 to 1.43)	<0.001
Log_2 (TCDD) within Ranch Hand cohort	1.19 (1.11 to 1.26)	<0.001	1.09 (1.02 to 1.17)	0.010	1.07 (1.00 to 1.15)	0.054
Comparison coefficient vs Ranch Hand coefficient‖		0.002		0.002		0.006

*Adjusted only for year of birth.

†Adjusted for year of birth, military occupation, race, age and BMI at the end of service in Southeast Asia, and family history of hypertension. Stratified by year of birth in 5 year intervals.

‡Adjusted for year of birth, military occupation, race, age and BMI at the end of service in Southeast Asia, family history of hypertension, alcohol drink-years to 1982, smoking pack-years to 1982, change in body mass index from the end of service in Southeast Asia to the TCDD measurement, and lipids (total cholesterol, HDL cholesterol and triglycerides in the serum used for the TCDD measurement). Stratified by year of birth in 5 year intervals.

§ 0=Comparison, 1=Ranch Hand. Within-group coefficients are unequal. Between-group comparison occurred at log (base 2) TCDD =2, equivalent to TCDD=4 ppt.

‖ Contrast of coefficients of Log_2(TCDD) within the Ranch Hand cohort and Log_2(TCDD) within the Comparison cohort.

We conducted additional secondary analyses to assess more closely the role of confounding in these data. Table 7 displays some of the covariate patterns across categories of TCDD within the Comparison and Ranch Hand cohorts. Generally, age at the end of service in SEA increased with TCDD except for the highest two TCDD categories in the Ranch Hand cohort. The highest two Ranch Hand categories were predominantly comprised of enlisted personnel. Within the Ranch Hand cohort, the percentage of veterans reporting a family history of hypertension increased with TCDD, regardless of whether the veterans had a history of hypertension. BMI at the end of service in SEA increased monotonically with TCDD category in both cohorts, despite the fact that veterans in the highest two categories of TCDD in the Ranch Hand cohort were relatively young. The relation between BMI at the end of service in SEA and categories of TCDD within each cohort are shown in Figure 2.

Table 7. Covariates with strong confounding potential by cohort and level of TCDD.

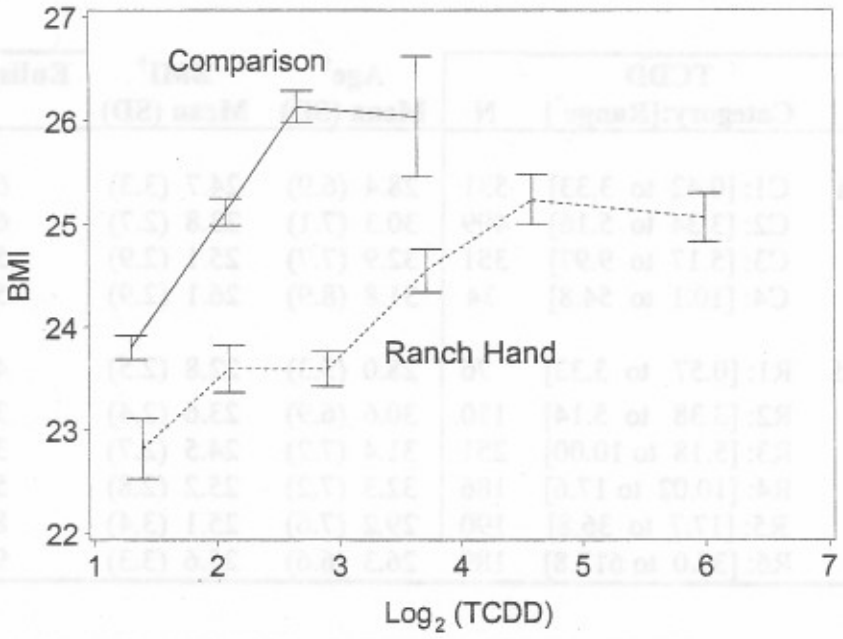
Cohort	TCDD Category:[Range*]	N	Age† Mean (SD)	BMI† Mean (SD)	Enlisted (%)
Comparison	C1: [0.42 to 3.33]	531	28.4 (6.9)	24.7 (3.3)	65.5
	C2: [3.34 to 5.16]	499	30.3 (7.1)	23.8 (2.7)	62.5
	C3: [5.17 to 9.97]	351	32.9 (7.7)	25.1 (2.9)	55.8
	C4: [10.1 to 54.8]	34	31.8 (8.9)	26.1 (2.9)	55.9
Ranch Hand	R1: [0.57 to 3.33]	76	28.0 (5.3)	22.8 (2.5)	48.7
	R2: [3.38 to 5.14]	110	30.6 (6.9)	23.6 (2.4)	35.5
	R3: [5.18 to 10.00]	251	31.4 (7.2)	24.5 (2.7)	39.4
	R4: [10.02 to 17.6]	186	32.3 (7.2)	25.2 (2.8)	53.2
	R5: [17.7 to 36.8]	190	29.2 (7.6)	25.1 (3.4)	86.3
	R6: [37.0 to 617.8]	189	26.3 (6.6)	25.6 (3.3)	99.5

			Diagnosis of hypertension?			
			No		Yes	
Cohort	TCDD Category:[Range*]	N	N	Family History of Hypertension (%)	N	Family History of Hypertension (%)
Comparison	C1: [0.42 to 3.33]	531	362	28.5	169	45.0
	C2: [3.34 to 5.16]	499	289	26.3	210	34.8
	C3: [5.17 to 9.97]	351	171	25.7	180	32.2
	C4: [10.1 to 54.8]	34	13	15.4	21	52.4
Ranch Hand	R1: [0.57 to 3.33]	76	60	18.3	16	12.5
	R2: [3.38 to 5.14]	110	76	26.3	34	38.2
	R3: [5.18 to 10.00]	251	152	25.7	99	34.3
	R4: [10.02 to 17.6]	186	109	27.5	77	36.4
	R5: [17.7 to 36.8]	190	99	36.4	91	39.6
	R6: [37.0 to 617.8]	189	101	46.5	88	44.3

*Parts per trillion

† At end of service in Southeast Asia.

Figure 2. Body Mass Index at tour versus TCDD category median by exposure cohort



LEGEND: The median of the logarithm base 2 of TCDD within each TCDD category is plotted on the horizontal axis. The mean body mass index (BMI) at the TCDD measurement (kg/m^2) is plotted on the vertical axis. Error bars extend to the mean plus or minus one standard error of the mean.

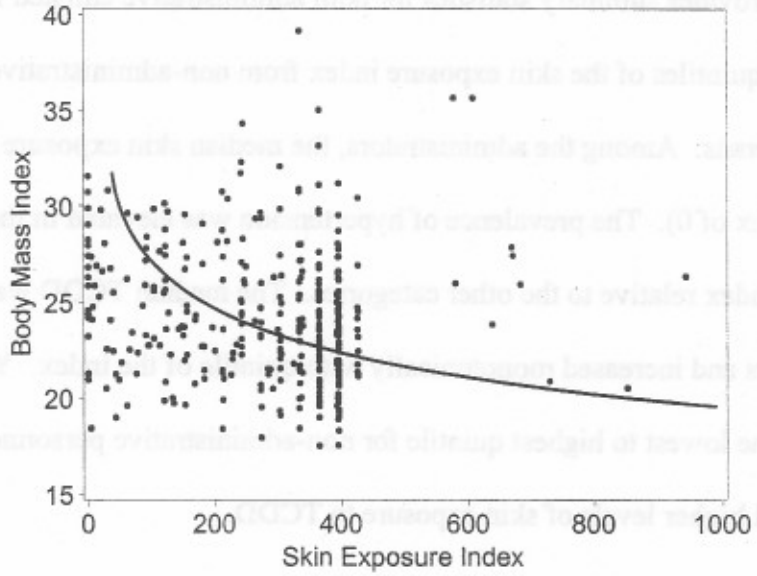
Restricting to enlisted Ranch Hand veterans with a non-missing herbicide skin exposure index (N=549), we regressed the $\log_2$ (TCDD) on military occupation, the logarithm of [1 + (skin exposure index)/100], BMI and the square of BMI at the end of service in SEA. Table 8 summarizes results for the regression model. Figure 3 shows a curve representing a constant value of TCDD (in this case the median of 29.0 ppt) based on results from the regression model overlaid on a scatter plot of BMI versus total days of skin exposure for these veterans.

Table 8. Coefficients for regression of logarithm (base 2) TCDD on occupation group, the logarithm of [1+ (skin exposure index)/100], BMI at the end of service in Vietnam, and the square of BMI at the end of service in Vietnam among Ranch Hand enlisted personnel (N=549).

Variable	Regression Coefficient (Std Error)	p-value
Intercept for enlisted flyers	-9.101 (2.482)	<0.001
Intercept for enlisted ground crew	-8.313 (2.464)	<0.001
Skin exposure index*	1.010 (0.121)	<0.001
Body mass index†	0.783 (0.193)	<0.001
BMI† squared	-0.012 (0.004)	0.002

*Log transformed, after dividing by 100 and adding 1. Skin exposure was calculated as total number of days of the Vietnam tour during which herbicide came into contact with the skin based on answers to a questionnaire detailing the number of months each duty was performed and how often it was performed during each month.
†At the end of service in Southeast Asia.

Figure 3. Body Mass Index at tour versus reported total days of skin exposure to TCDD in enlisted ground veterans



LEGEND: The skin exposure index is plotted on the horizontal axis. The mean body mass index (BMI) at the TCDD measurement (kg/m^2) is plotted on the vertical axis. The curve represents values of days of skin exposure and BMI where TCDD is held constant at 28.0 ppt in the fitted equation $\log_2(TCDD) = -8.313 + 1.010 \times \log(1 + \text{total skin exposure}/100) + 0.783 \times BMI - 0.012 \times BMI^2$.

Table 9 provides summary statistics for both administrative enlisted Ranch Hand veterans and for quintiles of the skin exposure index from non-administrative enlisted Ranch Hand veterans. Among the administrators, the median skin exposure index was 0 (64% had an index of 0). The prevalence of hypertension was elevated in the 1st and 5th quintiles of the index relative to the other categories. The median TCDD was lowest for the administrators and increased monotonically with quintile of the index. Year of birth increased from the lowest to highest quintile for non-administrative personnel, as younger veterans reported higher levels of skin exposure to TCDD.

Table 9. Quintiles of skin exposure index* and selected statistics in 549 enlisted Ranch Hand veterans.

Skin Exposure Index*				
Quintile	Median [Range]	As a Percent of Total Days of Tour (Mean)	Percent with Index=0	N
Administrative personnel§				
	0 [0 to 396]	18	64	53
Non-administrative personnel§				
1	35 [0 to 121]	16	30	97
2	181 [122 to 245]	56	0	101
3	304 [247 to 339]	83	0	101
4	365 [340 to 395]	94	0	105
5	396 [396 to 972]	96	0	92

Skin Exposure Index*							
Quintile	Median [Range]	N	Hypertension (%)	TCDD† (Median)	Year of Birth Mean (SD)	BMI‡ Mean (SD)	
Administrative personnel§							
	0 [0 to 396]	53	36	7.8	1940.2 (5.6)	25.4 (3.2)	
Non-administrative personnel§							
1	35 [0 to 121]	97	51	15.3	1937.0 (7.9)	24.9 (3.0)	
2	181 [122 to 245]	101	38	20.3	1939.7 (8.4)	24.9 (3.4)	
3	304 [247 to 339]	101	38	29.5	1941.6 (7.3)	24.5 (3.5)	
4	365 [340 to 395]	105	35	31.6	1942.5 (6.2)	23.8 (3.1)	
5	396 [396 to 972]	92	49	33.1	1942.4 (7.1)	24.3 (3.4)	

* Skin exposure was calculated as total number of days of the Vietnam tour during which herbicide came into contact with the skin based on answers to a questionnaire detailing the number of months each duty was performed and how often it was performed during each month.

†Parts per trillion.

‡ At the end of service in Southeast Asia.

§ Administrative status determined by a manual review of military records. Of the 53 administrative Ranch Hand veterans, 34 (64%) reported the number of skin exposure days as 0.

Table 10 summarizes the relative risk of hypertension by quintile of the skin exposure index relative to the 1st quintile for each of three statistical models: 1) adjusted only for year of birth; 2) adjusted for year of birth, military occupation, race, age and BMI at the end of service in SEA, family history of hypertension, and the skin exposure index as a percent of total days in SEA; and 3) adjusted for year of birth, military occupation, race, age and BMI at the end of service in Vietnam, family history of hypertension, the skin exposure index as a percent of total days in Vietnam, drink-years, pack-years, change in BMI from the end of service in SEA to the TCDD measurement, and lipids (total cholesterol, HDL cholesterol and triglycerides). After adjustment for covariates in Models 2 and 3, the risk of hypertension in the 5th quintile of the index was significantly increased relative to the 1st and there was a significantly increasing trend across the quintiles.

Table 10. Relative risk of hypertension for non-administrative Ranch Hand enlisted personnel by quintile of the skin exposure index relative to the first quintile of the skin exposure index.

Skin Exposure Index* Quintile:[Range]	Model 1* RR (95% CI)	Model 2† RR (95% CI)	Model 3‡ RR (95% CI)
1: [0 to 121]	Reference	Reference	Reference
2: [122 to 245]	0.81 (0.52 to 1.25)	0.96 (0.57 to 1.62)	1.08 (0.63 to 1.83)
3: [247 to 339]	0.92 (0.60 to 1.42)	1.26 (0.66 to 2.42)	1.39 (0.71 to 2.71)
4: [340 to 395]	0.81 (0.52 to 1.26)	1.34 (0.64 to 2.78)	1.57 (0.74 to 3.33)
5: [396 to 972]	1.34 (0.88 to 2.03)	2.15§ (1.04 to 4.47)	2.62§ (1.24 to 5.52)
Trend‖	p=0.056	p=0.006	p=0.002

*Adjusted only for year of birth.
†Adjusted for year of birth, military occupation, race, age and BMI at the end of service in Vietnam, family history of hypertension, and the skin exposure index as a percent of total days in Vietnam
‡Adjusted for year of birth, military occupation, race, age and BMI at the end of service in Vietnam, family history of hypertension, the skin exposure index as a percent of total days in Vietnam, alcohol drink-years to 1982, smoking pack-years to 1982, change in body mass index from the end of service in Vietnam to the TCDD measurement, and lipids (total cholesterol, HDL cholesterol and triglycerides in the serum used for the TCDD measurement).
§ Significantly different from 1.0 ($p<0.05$).
‖ P-value of linear trend test on relative risks from Cox model.

DISCUSSION

The current investigation found serum TCDD was significantly and adversely related to the risk of hypertension within both the Comparison and Ranch Hand cohorts. This pattern was first noted with a simple dichotomy of TCDD to less than or greater than 5 ppt and, subsequently, with more detailed TCDD categorizations within each cohort. The dichotomous TCDD analysis revealed significantly increased relative risks within each group, and, ignoring TCDD, no significant difference in risk between groups. This phenomenon is known as Simpson's Paradox and has been described as an artifact of confounding[31]. We attempted to identify a confounder that will satisfactorily explain this pattern. To that end we studied the effects of BMI, age, family history of hypertension, and other known risk factors. In addition, we considered skin exposure to herbicides among Ranch Hand enlisted veterans, indexed by days of skin exposure while on the job in Vietnam during the war, derived from questionnaire responses obtained without the veterans' knowledge of TCDD levels.

Within the Ranch Hand cohort the relative risk of hypertension increased with increases in a skin exposure index. This relation was not explained by differences in lifestyle (such as alcohol consumption), family history of hypertension, BMI at the end of service in SEA, changes in BMI, or serum Triglycerides levels, although all of these contributed significantly to the model. A possible explanation for this pattern is that tissue TCDD level is indicative of metabolic factors that are causal in the development of hypertension. For example, TCDD level may be reduced by elevated levels of detoxifying factors or expression of genes for the production of detoxifying factors. These same factors may prevent the development of

hypertension via several pathways. Thus, an elevated tissue level of TCDD (relative to exposure) may be an indicator of reduced capacity for clearance of toxins, and this reduced capacity could then promote the development of cardiovascular pathology[32] including hypertension. Alternatively, the trends within groups could be spurious, the result of confounding by an as yet unidentified factor.

The relationships between TCDD and other factors, such as the skin exposure index, occupation group, and BMI at the end of service in SEA, strongly suggest that the level of TCDD is a function of both level of exposure to TCDD and physiological parameters such as BMI. This would provide an explanation for why the relative risk of hypertension for a given level of TCDD appeared to be higher for the Comparison veterans in Figure 1. A Ranch Hand veteran with the same TCDD level as a Comparison veteran was likely to have had a greater exposure to TCCD in SEA but a lesser BMI. Figure 3 supports this interpretation, because the model suggested that TCDD was determined by both BMI and skin exposure among enlisted Ranch Hand veterans. For example, two veterans with the same TCDD level could differ with regard to BMI and skin exposure, with one having a greater BMI and lesser skin exposure and the other a lesser BMI and greater skin exposure. It was for this reason that we did not attempt to model hypertension in terms of TCDD in the combined cohort.

The strengths of the age-adjusted relations between serum TCDD and the risk of hypertension suggest that TCDD level is closely related to some factor that has a strong influence on the pathophysiology of hypertension. The strong association between TCDD and

BMI suggests that TCDD level is also closely associated with factors determining body weight. Several mechanisms are plausible: increased body fat could increase uptake of toxins from exposure; increased body fat could also be associated with metabolic factors that reduce detoxification; and body weight could be regulated upward in a systemic effort to dilute lipophilic toxins. The substantial attenuation in the relation between serum TCDD and hypertension with adjustment for BMI and lipids makes a spurious explanation of the TCDD link with hypertension plausible, however, an adverse relation between hypertension and the herbicide skin exposure index, argues against this interpretation.

It is plausible, that a metabolic syndrome (resulting in elevated BMI and triglycerides[33]) both promotes hypertension and reduces the capacity of detoxifying enzymes to remove TCDD and related compounds from tissue and blood. This hypothetical syndrome may be promoted by TCDD and other environmental toxins and may alter clearance of TCDD-related compounds and other toxins from tissue and blood[34,35]. In this case, the relation observed between serum TCDD and hypertension risk within cohorts may be due both to confounding and a causal effect of toxins on the etiology of the metabolic syndrome and hypertension. But, once again, the adverse relation between hypertension and the skin exposure index agues against a spurious result caused by confounding. Further resolution of these interpretations does not appear possible with available data.

The strengths of this study include high participation rates, a Comparison cohort closely matched to the index cohort, the presence of the TCDD-blinded skin exposure index, and 15 years of follow-up. Repetitive examinations and active quality control incorporating double

blind entry of data with discordances referred for third-party review reduced errors that would have biased the study toward the null result. At the first physical examination, participants delivered all medical records since service in Vietnam and at subsequent examinations all records since the previous examination. Participants who reported taking anti-hypertensive medications were asked to provide medical records from the prescribing physician. These record reviews reduced the likelihood that cases of hypertension were missed. We included models that adjusted for risk factors measured prior to, or very near, the exposure, such as year of birth and BMI during service in SEA to avoid the possibility of bias induced by covariates that may have been influenced by the exposure[36]. In addition, we included models that adjusted for other potential confounders that could not be measured prior to the start of the follow-up period, such as pack-years of smoking up to 1982. We avoided using covariates measured concurrent with or after the hypertension diagnosis.

The study was limited because TCDD's health effects are mediated via the Ah receptor, and other compounds related to TCDD also act via the Ah receptor and potentially affect disease risk. TCDD and these other compounds, i.e., the other polychlorinated dibenzo-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls, are present in the food supply in developed countries and most people have detectable levels[1]. In people with no unusual exposure to TCDD, only about 10% of the Ah receptor binding activity of this group of compounds is due to TCDD[34, 35]. Our focus on TCDD levels may overstate the effect of exposure (via Ah receptor activation). While the presence of these other compounds has no immediate effect on the interpretation of our findings, their existence is of note in the event that a precise estimation of dose-response is attempted. Our decision to measure only TCDD was made before it was known that other related compounds also activate the Ah-receptor. As

already mentioned, these results could be biased by lack of adjustment for other risk factors unknown to us and that we have not measured. Our findings may be of general methodologic interest, since they demonstrate how use of a biomarker within a population may be misleading about effects of exposure. Associations between biomarker levels and risk of disease may reflect detoxifying factors rather than exposure to the toxin of interest. This source of confounding can be minimized by using biomarkers to calibrate other exposure measures that are not subject to such confounding[18].

In conclusion, the risk of hypertension increased with TCDD in both cohorts and, without regard to TCDD, the risk was not increased in the Ranch Hand cohort, phenomenon known as Simpson's Paradox. Adjustment for risk factors, stratification by TCDD level, and the replacement of TCDD by reported skin exposure to herbicides revealed a consistent pattern of increased risk in each cohort, but did not reveal a significant difference in risk between cohorts without regard to TCDD exposure. These patterns may be related to as yet unidentified metabolic processes or may be entirely spurious. We have not been able to identify sources of bias or confounding that could fully account for the relation we found between TCDD and hypertension or between skin exposure to herbicides and hypertension. Thus, our findings suggest that metabolic processes involved in TCDD uptake and clearance, such as production of detoxifying enzymes, are closely linked to the pathophysiology of hypertension and change in body weight.

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